REMARKS

The claims are 1, 2, 7-18, 20-25, 30 and 34, of which Claims 1, 2, 11 and 34 are in independent form. Reconsideration of the present claims is respectfully requested.

In the Office Action, Claims 1, 2, 7-18, 20-25, 30 and 34 are again rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over *Winskill et al.*, Animal Behavior Science 48:25-35 (1996) in view of *Johnson et al.*, Equine Veterinary Journal 30(2): 139-143 (1998), further in view of Pagan, Australian Equine Veterinarian 16(4): 159-161 (1998). Claims 24 and 25 are likewise again rejected under § 103(a) as allegedly unpatentable over *Johnson et al.*, and *Winskill et al.*, in view of *Pagan.* Applicants respectfully traverse these rejections.

Applicants once again gratefully acknowledge the Examiner's indication that Claims 20 and 21 would be allowable if rewritten in proper independent forum. However, these claims will not be so rewritten for at least the following reasons.

In response to Applicants' previously-submitted arguments regarding the percentages of fat and fiber, the Examiner concedes that "2.75% fat is lower than the about 5% minimum in the fat recited in the claim 1", but nonetheless alleges that "[t]here is no demonstration that a composition comprising about 2.75% fat and 20% fiber would not alleviate stereotypic behavior in the presence of an antacid." Office Action, page 8.

Initially, Applicants believe it necessary to reiterate that the about 5% to 20% range for the fat recited in Claim 1 is critical for proper function of the present invention.

In support of their position, Applicants respectfully wish to first point out that, as relevant to the present invention, feeding of concentrate diets (either commercial

feeds or cereal-based home mixes) to foals has been shown to increase markedly the risk of development of stereotypy (*See* e.g., Waters A.J, Nicol C.J., French N.P. "*Factors influencing the development of stereotypic and redirected behavior in young horses: the findings of a four year prospective epidemiological study*", Eq. Vet. J. 34, 572-579 (2002)). In 2001, available concentrate diets for mares and youngstock typically had fat/oil levels much less than 5%. Evidence of this is provided in the enclosed paper by Williams et al., "*Plasma glucose and insulin responses of Thoroughbred mares fed a meal high in starch and sugar or fat and fiber*", American Society of Animal Science, pages 2196-2201 (2001). In particular, Table 2 of this paper shows that Pelleted concentrate feed, PurePride-200 (PC, Purina Mills, St Louis, MO - page 2197, left column second paragraph - the main brand in the US at the time) contained only 1.97% fat. Meanwhile, a control diet (SS) formulated to resemble closely a traditional textured sweet feed high in sugar and starch contained 3.2% fat.

Oats and maize are typically used in cereal-based diets. On average they contain 4% oil on a DM basis (*see* Lonsdale C., "*Straights: raw materials for animal feed compounders and farmers*", Chalcome publications, Marlow, Bucks UK (1989)), but only 3.4% on an as fed basis (*see* enclosed Dodson & Horrell Yearling Cubes & Rearing Diet).

The composition of the diet used as the control base diet (*see* specification, page 10, line 27) in the present application also has less than 5% fat (*see* the enclosed nutrient analysis for Dodson & Horrell Yearling Cubes & Rearing Diet). This diet was a leading brand in the UK at the time. Thus, the foregoing evidence makes clear the link between stereotypic behavior and concentrated diets of less than 5% fat.

To illustrate the advantageous effect of using feed containing the critical range of a minimum 5-20% fat as recited in Claim 1 for overcoming the above-noted dietary deficiencies, Applicants kindly draw the Examiner's attention to the enclosed abstract, Geor R J, Harris PA, Hoekstra KE, Pagan J D, "*Effect of corn oil on Solid phase gastric emptying in horses*," Proceedings of the ACVIM no 67 p 288, (2001), describing a study in which it is concluded that addition of 10% corn oil to a meal sweet feed results in a delay in solid-phase gastric emptying. The delay in gastric emptying is believed to prolong the beneficial effect of the antacid and fiber of the composition.

Moreover, the upper limit of 20% fat is specified in the composition of Claim 1 because it is known in the art that horses can eat up to 20% fat in their diet without adverse effects (*see*, e.g., Harris P & Kronfeld, "*Influence of dietary energy sources on Health and performance*", Current therapy in equine medicine 5, Robinson NE (ed) Saunders Philadelphia 698-704 (2003)).

Applicants also note that many different studies have shown that the risk of crib-biting in horses is increased by low forage (i.e., low fiber) or high starch diets including, for example:

- Bachmann, I., Audige, L., Stauffacher, M., "*Risk factors associated with behavioural disorders of crib-biting, weaving and box-walking in Swiss horses*", Eq. Vet. J. 35, 158-163 (2003);

- McGreevy, P., Nicol, C.J., Cripps, P., Green, L., French, N., "*Management factors associated with stereotypic and redirected behaviour in the thoroughbred horse*", Eq. Vet. J. 27, 86-91 (1995);

- Redbo L,. Redbo-Torstensson P, Odberg F.O,. Hedendahl A,. Holm J., "*Factors affecting behavioural disturbances in race-horses*", Anim. Sci,. 66, 475-481 (1998);

- Waters A.J, Nicol CJ, French N.P., "*Factors influencing the development of stereotypic and redirected behaviour in young horses: the findings of a four year prospective epidemiological study*", Eq. Vet. J. 34, 572-579 (2002).

With a specified minimum amount of 5%, the amount of fiber recited in the composition of Claim 1 is believed to be higher than in a low forage diet. For example, a typical neutral detergent fiber composition of maize (a low fiber feed) is about 11% (*see* Lonsdale C., "*Straights: raw materials for animal feed compounders and farmers.*" Chalcome publications, Marlow, Bucks UK (1989)).

In addition, Applicants have themselves performed a study showing the effect of adding increasing percentages of chopped fiber (chaff) to the diet on the time it takes to eat a Kilogram of feed. The results are shown in the enclosed Figure 1, clearly evidencing that adding chopped fiber to the feed increases the time taken to eat the feed. The longer the time taken the more saliva is believed to be produced. This increased saliva is expected to assist in neutralizing stomach acidity.

Applicants further submit that there was no reason for one of ordinary skill to try to optimize the known compositions in order to treat animal stereotypy because there is no disclosure in either *Johnson* or *Winskill* that such compositions as recited in Claim 1 could be used to alleviate stereotypy. Respectfully, Applicants submit that the proper inquiry is whether or not it would have been obvious to one of ordinary skill to provide a composition within the scope of Claim 1 on the basis of the disclosure of the cited prior art. "The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure." MPEP § 2142 at 2100–128 (citing *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)). Because there is no disclosure of a link between stomach antacid

and performance of stereotypies in the cited references, there was no reason for one of ordinary skill to modify known compositions to provide optimized compositions that control stomach pH.

Applicants respectfully believe the above support illustrates precisely how a composition diet of about 5% to about 20% fat alleviates stereotypic behavior. To summarize, the at least 5% fat content delays gastric emptying, thereby prolonging the beneficial effects of the antacid and fiber. The chopped fiber, meanwhile, increases the eating time so that additional saliva is produced, thus further neutralizing stomach acidity.

It is respectfully submitted that the amount of fat and fiber recited is, therefore, critical for the composition of Claim 1 to have a significant effect on the treatment or amelioration of animal stereotypy, or for minimizing the risk of an animal developing animal stereotypy. Applicants have also demonstrated the beneficial effect of use of chopped fiber for this end. It is likewise clear from the above evidence that compositions outside of the scope of claim 1 would be expected to increase the risk of development of animal stereotypy. That is, by disclosing only a 2.75% fat content, *Johnson* fails to recognize the critical minimum 5% amount necessary to decrease the incidence of stereotypy and, consequently, precludes a finding of obviousness in regard to the present application. Claim 1 is therefore believed patentable over *Johnson* in combination with the other applied art.

The Examiner goes on to comment that Applicants' "assertion that fat and fiber content critical to alleviating stereotypy appears not to reflect the method claim 11." Office Action, page 9. Applicants respectfully disagree. Claim 11 is directed to a method of treatment or amelioration of animal stereotypy, or of minimizing the risk of an animal developing animal stereotypy, by controlling stomach pH of the animal. The composition

outlined in Claim 1 is designed specifically to control stomach pH. Page 19, lines 22-27 of the specification indicate that fiber is believed to help prolong chewing time. As explained above, this in turn prolongs the production of saliva which plays a role in neutralizing stomach acid. Use of chopped fiber is believed to further increase this effect. The fat, meanwhile, is thought to delay emptying of the stomach so that the beneficial effect of the antacid and fiber is prolonged. Accordingly, there is no inconsistency between the critical percentages recited in Claim 1 and the method as outlined in Claim 11.

The Office Action further indicates that *Johnson* is relied upon for a disclosure of feed containing antacid that lowers incidence of stereotypy in horses, and alleges that "the combination of Winskill and Johnson produces a food composition that is within the scope of instant claim 1." Office Action, page 9.

However, *Johnson* does not disclose administration of feed containing stomach antacid. Instead, the reference discusses only dietary supplementation with virginiamycin to reduce fermentative acidosis in the hindgut. As such, there is no teaching in this document that would lead one of ordinary skill to replace virginiamycin with a stomach antacid. "In determining the differences between the prior art and the claims, the question under 35 U.S.C. 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious." MPEP § 2141.02 at 2100–124 - 125 (citing *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983) (emphasis in original)).

The Examiner states that *Winskill's* feed composition, despite containing no antacid, was effective in combination with the foodball to increase foraging time and thus lowered stereotypy. The Examiner then comments that a composition can be formulated

from the combination of *Winskill* and *Johnson* as a feed for horses, and the motivation of including the antacid of *Johnson* is to produce a feed that would be expected to lower the incidence of stereotypy.

However, a combination of the feed of *Winskill* with the antacid of *Johnson* (i.e., virginiamycin) does not produce a composition within the scope of the composition specified in Claim 1 and moreover, the composition formed would not be expected to control stomach pH, but rather hindgut acidity alone. If, on the other hand, the Examiner is instead referring to sodium carbonate as the antacid disclosed in *Johnson*, one of ordinary skill would not have combined this with feed because sodium carbonate is administered to the caecum to neutralize hindgut acidity (page 139, right column, last sentence of second paragraph), and so must be administered separately from the feed.

Applicants respectfully believe the Examiner to be combining selected passages from *Johnson* and *Winskill* in hindsight without any regard to the principal teaching in those documents. Such combination is not proper because, as indicated in the MPEP, "impermissible hindsight must be avoided and the legal conclusion must be reached on the basis of the facts gleaned from the prior art." MPEP § 2142 at 2100–128.

The Office Action further states there to be an expectation of success that stereotypy in the horse will be lowered when an antacid is included in the composition of *Winskill*, and therefore, allegedly, "it is *prima facie* obvious to substitute one antacid for another and expect to produce the same effect of acidity control." Office Action, page 9, 10. Respectfully, Applicants believe this conclusion ignores the crucial anatomical difference between the hindgut and the stomach.

At best, *Johnson* merely teaches a link between hindgut acidity and abnormal behaviour. Importantly, there is no disclosure of any link between stomach acidity and stereotypy as contemplated by the present invention. According to *Johnson*, hindgut acidity is controlled by one of two means: oral administration of virginiamycin or caecal administration of sodium carbonate. It is respectfully submitted, however, that substituting one antacid for another would not be *prima facie* obvious because some antacids will not be effective at controlling hindgut acidity if administered orally, and some antacids are only effective in certain tissues. Virginiamycin is limited to hindgut activity, while sodium carbonate can only be administered caecally. "If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification." MPEP § 2143.01 at 2100–132 (citing *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984)). Consequently, neither combination would operate to lower the incidence of stereotypy according to the mechanism of the present claims and, therefore, Applicants submit that Claim 1 is not obvious in view of the combination of *Johnson* and *Winskill*.

In support of their argument, Applicants wish to highlight, for example, histamine type-2 antagonists which block histamine-stimulated gastric acid secretion and so are expected to act as stomach antacids, but not to have any significant effect in the hindgut. Virginiamycin is active against bacteria that produce lactic acid in the hindgut, and so is expected to reduce fermentative acidosis in the hindgut, but not to have any significant effect on stomach acidity.

Recognizing the difference between these two separate organs based on his/her veterinary experience, one of ordinary skill in the art would not have thought to

administer sodium carbonate or sodium bicarbonate orally to cause a reduction in hindgut acidity. To have an effect on the hindgut, high doses of these compounds would be expected to be required. Yet, orally administering high doses of compounds such as sodium bicarbonate would at the same time likely produce a number of adverse effects, since the dietary acid/base balance of the diet would be significantly altered. This, in turn, can affect blood electrolyte levels, respiratory rate and the pH of the blood. For instance, oral administration of 0.5-1g/kg body weight sodium bicarbonate would cause significant metabolic acidosis. Indeed, Applicants believe that in certain states the addition of sodium bicarbonate beyond a certain amount is banned in racing because this is thought to have the potential to affect performance. Generally, high doses of sodium carbonate would be expected to cause gastric irritation, and therefore would not likely be used. As far as the Applicants are aware, sodium carbonate is not administered orally to horses.

The enclosed paper by Deuel et al., "*Some Physiological Effects of Sodium Bicarbonate in Diets of Yearling Horses*," Proceedings of the 7th Equine Nutrition and Phsiology Symposia, Virginia, USA, describes a study of the physiological effects of adding sodium bicarbonate to growth diets for yearling Quarter Horses. Addition of 1% sodium bicarbonate was found to be negatively correlated with fecal pH (page 21, lines 7-8 and Table 2). Specifically, in the Discussion on page 21 it is stated that "it has been observed that the pH of terminal colon digesta is similer to that of cecal contents ... the reduced pH of colon digesta in this study may have been associated with a reduced cecal pH". Thus, oral administration of sodium bicarbonate is thought to have increased, rather than decreased, hindgut acidity.

Consequently, one of ordinary skill in the art would not have substituted oral administration of virginiamycin or caecal administration of sodium carbonate (intended to control hindgut acidity) for oral administration of sodium carbonate or sodium bicarbonate as concluded in the Office Action. It is therefore respectfully submitted that substitution of one antacid for another with the expectation of the same effect on acidity control would not be *prima face* obvious to one of ordinary skill in the art.

At page 10, the Office Action indicates that "the references taken together disclose the composition of the instant claims and discloses also the method of the instant claims", and further alleges that "the combination [of references] is proper." Applicants, however, bring to the Examiner's attention MPEP § 2143 wherein it states that "[i]f the proposed . . . combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious." MPEP § 2143.01 at 2100-132 (citing *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959)).

Because neither *Johnson*, *Winskill* nor *Pagan* identifies the relationship between stereotypic behavior in animals and stomach acidity, any proposed combination thereof would likely fail to operate according to the teaching of the present claims. Specifically, a combination of these references would produce, at best, an unworkable treatment method in which a foodball is supplied to encourage foraging together with caecal administration of antacid for hindgut acidity. Respectfully, such combination is not the present invention, nor does it teach, or even suggest, either the composition as recited in Claim 1 or the method as recited in Claim 11. "To establish prima facie obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art."

MPEP § 2143.03 at 2100–133 (citing *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974)). Accordingly, Claims 1 and 11 are believed patentable over the combination of *Johnson*, *Winskill* and *Pagan*.

Based on all of the foregoing, Applicants respectfully submit that Claims 1 and 11 are patentable over the cited references, and earnestly request withdrawal of the rejection under 35 U.S.C. § 103(a).

Furthermore, the other claims in this application are each dependent from one or another of the independent claims discussed above and are therefore believed patentable for the same reasons. Since each dependent claim is also deemed to define an additional aspect of the invention, however, the individual consideration or reconsideration, as the case may be, of the patentability of each on its own merits is respectfully requested.

Wherefore, it is respectfully submitted that the cited art does not disclose or suggest the presently claimed invention, either alone or in combination. Accordingly, passage to issue of presently claimed invention is respectfully requested.

Applicants' undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should continue to be directed to our below listed address.

Respectfully submitted,

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DODSON & HORRELL

Horse Feed Specialists

YEARLING CUBES & REARING DIET

Yearling Cubes and its coarse equivalent Rearing Diet are formulations designed for fast growing young horses with the emphasis on good quality proteins and accurate levels of minerals and vitamins.

For the Thoroughbred, the aim is for optimum growth, muscle development and good bone density so vital at this critical stage when the first stresses of training are being experienced. Intake may vary between 6lbs - 10lbs per day according to the individual and up to 15lbs per day when being prepared for the sales.

For the non-Thoroughbred, Yearling Cubes and Rearing Diet (fed at reduced levels or according to individual requirements) will promote optimum bone and muscular development.

Mature horses lacking in condition may also benefit from a dietary course of Yearling Cubes or Rearing Diet, reverting to a working ration when the desired condition has been attained.

INGREDIENTS

YEARLING CUBES	REARING DIET
Cereals (Wheat Barley, Oats)	Micronized Flaked Soya
Wheatfeed	Micronized Flaked Peas
Soya Bean Meal	Micronized Flaked Barley
Peas	Micronized Flaked Wheat
Full Fat Linseed	Scotch Bruised Oats
Dicalcium Phosphate	Concentrate Pellet (including vitamins, mineral & protein sources)
Limestone	
Salt	Uniquely Blended Syrup
Vitamin & Mineral Supplement	
Molasses	

Guarantee

Dodson & Horrell Ltd wish to confirm that this horse feed does not contain any prohibited substances which may contravene the regulations of the F.E.I. and the Jockey Club of Great Britain.

RINGSTEAD NORTHAMPTONSHIRE
TELEPHONE: 0933-624221 FACSIMILE: 0933-625461

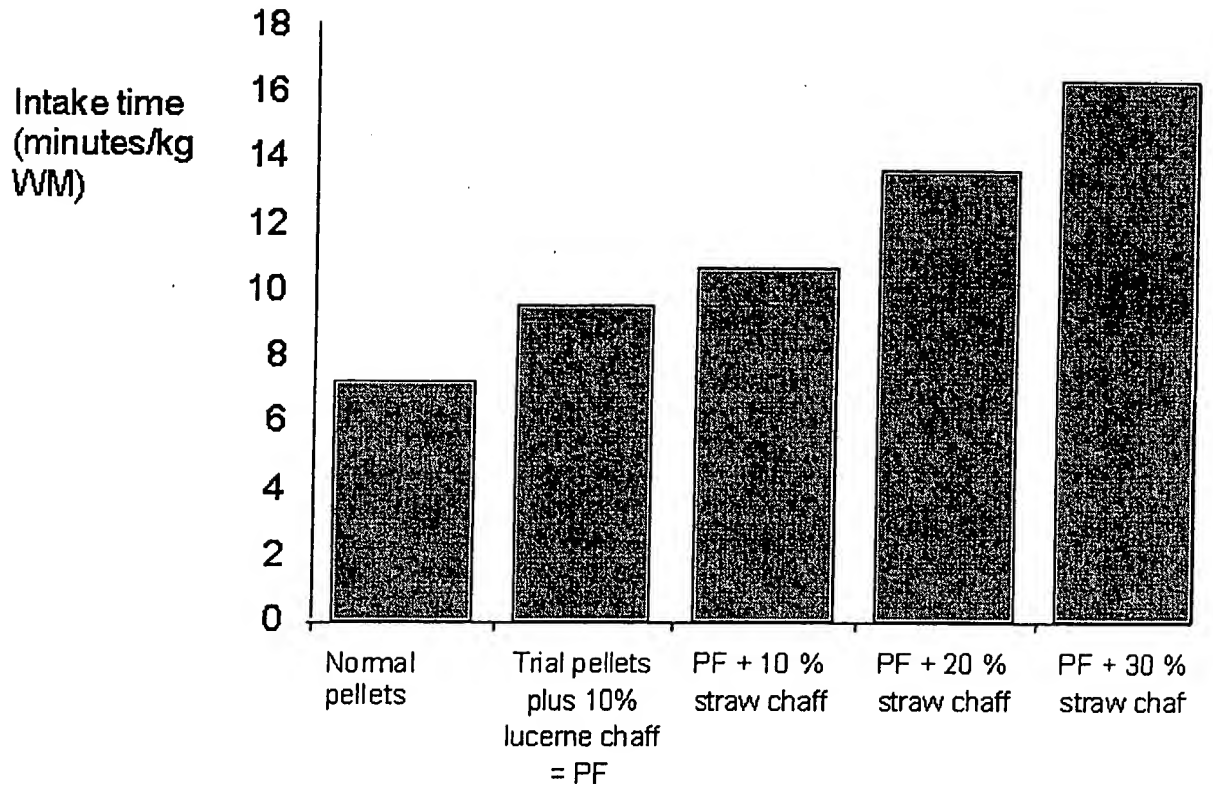
NUTRIENT ANALYSIS

YEARLING CUBES	
Protein	16.0% (160 g/kg)
Oil	3.7%
Fibre	9.0%
Ash	9.0%
Estimated Digestible Energy	12.0 MJ/kg
Calcium	1.3%
Phosphorus	0.7%
Salt	1.2%
Magnesium	1.9 g/kg
Lysine	0.75%
Vitamin A	14,000 iu/kg
Vitamin D_3	1,500 iu/kg
Vitamin E	200 iu/kg
Selenium	0.2 mg/kg
Copper	35.0 mg/kg
Zinc	85.0 mg/kg

REARING DIET	
Protein	16.0% (160 g/kg)
Oil	4.8%
Fibre	7.0%
Ash	7.0%
Estimated Digestible Energy	12.7 MJ/kg
Calcium	1.2%
Phosphorus	0.6%
Salt	1.0%
Magnesium	1.8 g/kg
Lysine	0.8%
Vitamin A	14,000 iu/kg
Vitamin D_3	1,500 iu/kg
Vitamin E	200 iu/kg
Selenium	0.2 mg/kg
Copper	35.0 mg/kg
Zinc	85.0 mg/kg

The vitamin and mineral supplement also supplies: Vitamins B_1, B_2, B_6, B_{12}, Biotin, Choline Chloride, Folic Acid, Pantothenic Acid, Nicotinic Acid, Vitamin K, Cobalt, Iodine, Iron, and Manganese.

Figure 1


Intake time (minutes/kg WM)
18
16
14
12
10
8
6
4
2
0
Normal pellets
Trial pellets plus 10% lucerne chaff = PF
PF + 10 % straw chaff
PF + 20 % straw chaff
PF + 30 % straw chaf

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65 THE EFFECT OF cAMP MODULATION ON EQUINE NEUTROPHIL ACTIVATION BY IMMUNE COMPLEXES.

C. D. Chilcott, Y. Sharief, S. L. Jones, Department of Clinical Sciences, North Carolina State University, Raleigh, North Carolina.

Toxic products such as reactive oxygen intermediates released by activated neutrophils (PMN) have an important role in the pathophysiology of diseases associated with the deposition of immune complexes (IC) in tissues. IC-induced activation of PMN requires adhesion mediated by integrin adhesion receptors. Of the integrins expressed on PMN, the β_2 family has been found to be of particular importance for activation of PMN by IC. β_2 integrin ligand binding must be activated to enable adhesion to IC. Both activating and inhibitory signals regulate β_2 integrin ligand avidity and adhesion. The second messenger cAMP has been demonstrated to inhibit the activation of PMN in response to a variety of stimuli. The purpose of this study is to test the hypothesis that cAMP-dependent signals inhibit β_2 integrin dependent adhesion of equine PMN to immobilized IC and subsequent adhesion-dependent activation of respiratory burst activity.

To test this hypothesis, we examined the effect of cAMP modulators on adhesion of purified equine PMN to immobilized IC using a 96 well microtiter plate adhesion assay. Fluorescently labeled PMN were allowed to adhere to immobilized IC or the control substrate serum. The fluorescence in each well was measured using a fluorescence plate reader before and after washing. Adhesion was quantitated as the percent PMN remaining after washing. Activation of respiratory burst activity during adhesion to IC or the control substrate serum was determined using a microtiter plate assay based on the measurement of the H_2O_2-dependent loss of scopoletin fluorescence.

Treatment of equine PMN with β_2 adrenergic agonists isoproterenol or clenbuterol, which trigger an increase in intracellular cAMP concentration, inhibited adhesion of equine PMN to IC in a dose dependent manner (isoproterenol IC50 ≈ 2.5mM, clenbuterol IC50 ≈ 150uM). Similarly, inhibition of cAMP metabolism by the non-specific phosphodiesterase inhibitor pentoxifylline and the phosphodiesterase 4-specific inhibitor rolipram inhibited adhesion of equine PMN to IC (pentoxifylline IC50 = [illegible], rolipram IC50 = [illegible]). Additionally, co-treatment of equine PMN with rolipram and isoproterenol synergistically inhibited the adhesion of equine PMN to IC. Modulation of intracellular cAMP levels also inhibited IC-induced activation of respiratory burst activity in equine PMN.

Our conclusion is that cAMP negatively regulates β_2-dependent adhesion of equine PMN to IC and subsequent activation of effector functions in horses. Thus, these drugs may be useful as anti-inflammatory agents in IC-mediated diseases. The synergy between β_2-agonists and phosphodiesterase inhibitors may also have clinical importance.

66 ENDOTOXIN-NEUTRALIZING ACTIVITY IN BLOOD AFTER INTRAVENOUS ADMINISTRATION OF POLYMYXIN B IN THE HORSE.

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Polymyxin B (PB) is a cyclic cationic polypeptide antibiotic that binds and neutralizes bacterial endotoxin (lipopolysaccharide; LPS). Administration of a single dose of PB has been shown to protect against effects of IV-administered LPS. In clinical situations, however, administration of the drug is often repeated several times. The purpose of this study was to determine endotoxin-neutralizing activity in blood after single and repeated doses of PB to establish a dosage schedule for PB in endotoxemic horses.

An assay for quantification of endotoxin-neutralizing activity in equine serum was developed. In brief, activity of samples was measured in vitro as ability to suppress LPS-induced production of NO (measured as NO_2^- concentration) by interferon gamma-primed cells of the J774A.1 murine macrophage cell line. Concentration of active PB in serum samples was determined by comparison with a standard curve of PB concentration vs. NO_2^- concentration generated using serial dilutions of PB in serum.

In a preliminary experiment, active (endotoxin-neutralizing) PB was measured in blood collected from 3 horses at intervals for 24 h after they were given a single IV dose of 1 mg PB sulfate/kg. PB was diluted in 1 L 0.9% saline and infused IV over 15 min. Maximal mean (± sem) serum concentration of active PB was 2955 ± 472 ng/ml 4 min after infusion and mean concentration declined to become undetectable by 16 h. Using Curry's method, these data were used to determine a dosage schedule of 1 mg PB/kg every 8 h. According to this schedule, trough concentration of active PB (> 200 ng/ml) were predicted to neutralize >75% of the NO_2^--inducing activity of 1 ng LPS/mL. Five adult horses each were given PB (1 mg/kg) IV every 8 h for 5 successive treatments and blood was collected for measurement of active PB concentration. Maximal mean (± sem) serum concentration of active PB was 3003 ± 910 ng/ml 10 min after the first infusion and declined to 250 ± 81 ng/ml at 7.75 h. The PB concentration profile after the 5th infusion did not differ significantly ($P < 0.05$, repeated measures ANOVA) from that for the first infusion. Maximal PB concentration was 2234 ± 1017 ng/ml, declining to 244 ± 100 ng/ml at 7.75 h, and to undetectable levels by 14 h post-infusion. Mean trough concentration after 5 infusions was 240 ± 14 ng/ml and trough concentrations did not differ significantly ($P < 0.05$). No abnormal clinical sign was seen during the experiment.

In conclusion, it appears that PB infused IV to horses at a dosage of 1 mg/kg every 8 h maintains adequate circulating anti-endotoxic activity without accumulation of drug or signs of toxicity.

67 EFFECT OF CORN OIL ON SOLID-PHASE GASTRIC EMPTYING IN HORSES.

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The ^{13}C-octanoic acid breath (or blood) test has been recently developed as a non-invasive method for measuring the rate of solid-phase gastric emptying (GE). We used this method to test the hypothesis that GE is delayed following ingestion of a grain plus corn oil meal compared to a meal of grain alone. Four mature (10–12 yr) Arabian horses were studied in a 2 x 2 factorial design. Factor A was the habitual diet, either a control (CON; hay plus sweet feed [SWF]) or an isocaloric fat-supplemented diet (FAT; hay, SWF and corn oil). Factor B was the type of meal consumed for the GE test (SWF, 2 g/kg bwt. vs. SWF 2 g/kg bwt. plus 10% corn oil [OIL]). Each diet period lasted 10 weeks, with 6 weeks in between. GE studies were performed during the 4th and 8th weeks in each period. Within each dietary period, and in random order, horses were tested in both the SWF and OIL conditions. The 4 treatment combinations being: CON/SWF, CON/OIL, FAT/SWF and FAT/OIL. For assessment of solid-phase GE, the test meals were labeled with 1 g of ^{13}C-octanoic acid. Blood samples for measurement of plasma glucose concentration and ^{13}C-enrichment were collected at 30 min and immediately before ingestion of the test meal and at frequent intervals thereafter for 7 h. Three indices of blood ^{13}C-enrichment were calculated: half-dose recovery time ($t_{1/2}$), the time to peak blood ^{13}C-enrichment (t(max)), and the gastric emptying coefficient (GEC).

The glycemic response was markedly decreased in the OIL compared to the SWF trials; this effect of corn oil was not altered by habitual diet. In 1 horse for both the CON/OIL and FAT/OIL trials, the blood ^{13}C vs. time curve was altered such that it was not possible to calculate $t_{1/2}$ and t(max). Excluding data from this horse, addition of corn oil to the meal of SWF was associated with a significant decrease in GEC and a significant increase in $t_{1/2}$ and t(max), as shown (mean ± s.d.):

Treatment	GEC	$t_{1/2}$ (h)	t(max) (h)
CON/SWF	2.96 ± 0.15	2.25 ± 0.55	1.20 ± 0.21
CON/OIL	2.10 ± 0.14	3.87 ± 0.39	2.08 ± 0.30
FAT/SWF	3.02 ± 0.09	2.21 ± 0.45	1.24 ± 0.37
FAT/OIL	2.03 ± 0.21	3.71 ± 0.66	2.19 ± 0.53

We conclude that: 1) the addition of corn oil to a meal sweet feed results in a delay in solid-phase GE; 2) the effect of oil on GE is not affected by short-term adaptation to a fat-supplemented diet; and 3) the slowing of GE may contribute to the blunted glycemic response following a grain meal containing corn oil. The delayed GE may be due to a direct effect of oil on motility or the resultant increased energy density of the test meal.

68 PREVALENCE OF GASTRIC ULCERS IN STANDARDBRED RACEHORSES IN QUEBEC.

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The purpose of this study was to determine the prevalence of gastric ulcers and to identify risk factors for their presence in Standardbred racehorses. Two hundred seventy-five (275) Standardbred racehorses from five training centers and two racetracks in Quebec, Canada, were included in the study. Historical data from the previous 2 months were recorded for each horse and the presence of gastric ulcers (GU) was determined by gastroscopy using a 3m videoendoscope. All evaluations were done from September to December, 1999. Location and severity (using a scoring system, grades 0 to 3) were recorded. The association between the presence of GU or score and a given risk factor was determined using univariate statistical analysis. Horses with GU (score 1–3) were compared to those without GU (score 0) using logistic regression analysis to identify risk factors. A significance level of $p < 0.05$ was used for analysis.

The study population was composed of 112 females (41%), 80 geldings (29%) and 83 stallions (30%). There were 160 pacers (58%), 105 trotters (38%) and 10 horses (4%) for which gait was unknown at the time of the data collection. Forty-four horses were at rest, 92 were in training and 139 were actively racing. Overall, 121 horses (44%) had GU while the prevalence of GU in actively racing horses was 63% (n = 88). The following factors were significantly associated with the presence of GU: activity status ($p < 0.0001$) for a horse in training (OR = 2.18) or racing (OR = 9.29), gait ($p = 0.004$) for trotters (OR = 2.23) and racetrack stables ($p = 0.0027$). The mean number of lesion sites ($p < 0.0001$) was significantly associated with the gastric lesion score. The only clinical sign significantly associated with the presence of GU was poor body condition ($p = 0.02$). Poor body condition was significantly associated with ulcers located at the cardia ($p < 0.0001$) and with lesions scores ≥ 2 ($p < 0.0001$).

The prevalence of GU in Standardbred racehorses in this study was slightly lower than in Thouroughbred racehorses (60–90%). Horses that are actively racing, are living at racetracks, are trotters or have poor body condition are more likely to have GU. Also, GU lesion score (grades 0–3) based on lesion size was found to also reflect the number of sites where lesions were found in the stomach. This further validates the scoring system with regards to its correlation with severity.

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Plasma glucose and insulin responses of Thoroughbred mares fed a meal high in starch and sugar or fat and fiber[1]

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ABSTRACT: Plasma concentrations of glucose and insulin following a meal were compared in twelve Thoroughbred mares fed a pelleted concentrate (PC), a traditional sweet feed high in sugar and starch (SS), or a feed high in fat and fiber (FF). The feeds had similar DE and CP but differed in fat (19, 32, and 166 g/kg DM, respectively), NDF (199, 185, and 369 g/kg DM, respectively) and nonstructural carbohydrates (574, 645, and 247 g/kg, respectively). Mares were randomly assigned to two groups balanced for foaling date and weight. All mares received PC in late gestation; then, after foaling, one group was fed SS and the other FF for trials in early and late lactation. Mares were placed in stalls and deprived of feed overnight. A series of blood samples was collected via a jugular catheter from 0 (baseline) to 390 min after consumption of 1.82 kg of feed. Plasma was analyzed for glucose and insulin. Baseline values, peak values, and areas under curves (AUC) were compared by ANOVA. Baseline values were 74.7 ± 10.9 mg/dL for glucose and 5.86 ± 1.80 mIU/L for insulin for all diets and stages. Responses to PC did not differ between the two groups ($P > 0.34$), indicating the groups were metabolically similar. Peak plasma glucose and insulin concentrations were higher ($P < 0.001$) in the SS group than in the FF group during early and late lactation. Similarly, glucose and insulin AUC were larger ($P < 0.003$) in SS than in FF during early and late lactation. These results indicate that metabolic fluctuations are moderated by the replacement of sugar and starch with fat and fiber. This replacement may reduce the risk of certain digestive and metabolic disorders that have been linked to feeding meals of grain-based concentrates to pregnant or lactating mares.

Key Words: Dietary Fat, Fiber, Glucose, Horses, Insulin

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Introduction

Concentrates are commonly fed as two meals a day to supplement forage (pasture and[or] hay) in the diet of horses, especially those with energy and nutrient requirements above maintenance. A meal containing a high percentage of a grain-based concentrate has been associated with digestive, circulatory, metabolic, and hormonal changes (Clark et al., 1990). Abrupt increases or high intakes of soluble carbohydrates may create large fluctuations in the growth curve (Hoffman, 1997) and increase the risk of certain digestive and metabolic disorders (Kronfeld, 1998). Pregnant or lactating mares have also been studied in terms of their glycemic response varying due to fetal drain and need for glucose in milk production during these stages (Evans, 1977; Fowden et al., 1984).

Replacement of starch with fat in the feed has been used in many areas of interest, some of which include preventing rhabdomyolysis (Kronfeld, 1973; Valentine et al., 1998; MacLeay et al., 1999), enhancing aerobic (Slade et al., 1975) and anaerobic exercise (Oldham et al., 1990), and reducing excitability (Holland et al., 1996). Our laboratory has been developing horse feeds in which sugar and starch are replaced with fat and fiber (Kronfeld, 1996; Hoffman et al., 1998; Hoffman and Kronfeld, 1999). The present study examined the glycemic and insulinemic responses to typical course grain or pelleted concentrates compared to a supplement formulated with fat, to provide the same energy density of a typical concentrate, and fiber, to allow its use as a complete feed.

Materials and Methods

A total of 12 Thoroughbred mares, aged 13.3 ± 4 yr, were used in the study. The protocol was approved by

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Table 1. Ingredient composition (%) of the sugar and starch (SS) and fat and fiber (FF) feeds

	Diet	
Ingredient, %	SS	FF
Dent yellow grain corn	60	11
Soybean meal (48% CP)	15.5	2
Oat straw	7	7
Alfalfa	0	13.5
Soybean hulls	4	4
Beet pulp	0	10
Processed cereal by-product[a]	0	45
Molasses (cane)	10	5
Dicalcium phosphate	1.5	0.5
Limestone	1	1
Mineral premix[b]	0.5	0.5
Vitamin premix[c]	0.5	0.5

[a]Processed cereal by-product contains 92.5% DM, 26% EE, 18% CP, and 29% NDF.

[b]The mineral premix provided the following per kilogram of supplement: NaCl, 3,774 g; Zn, 422 g; Fe, 208 g; Cu, 89.5 g; Mn, 50.3 g; Se, 1.095 g; and KI, 0.416 g.

[c]The vitamin premix provided the following per kilogram of a supplement: vitamin A, 1,350,060 IU; vitamin D_3, 258,000 IU; vitamin E, 26,455 IU; riboflavin, 701 mg; niacin, 3,009 mg; folic acid, 66 mg; thiamin, 1,400 mg; biotin, 42 mg; and β-carotene, 3,527 mg.

the institutional animal care and use committee and performed at the Virginia Tech Middleburg Agriculture Research and Extension Center.

The mares were maintained on adjacent bluegrass and white clover pastures with free access to alfalfa and orchardgrass hay. They were paired by weight and foaling date then randomly assigned to two groups as of January. For three months prior to foaling the pregnant mares were fed 3.5 kg/d of a pelleted concentrate (PC, PurePride-200, Purina Mills, St Louis, MO). The mares were introduced to the experimental feeds just after foaling; one group received a control diet (SS), which was formulated to closely resemble a traditional textured sweet feed high in sugar and starch, and the other group received a test feed high in fat and fiber (FF). The SS and FF feeds were formulated (Table 1) to be isocaloric, and their trace mineral and vitamin contents were balanced with the pastures to meet or exceed recommendations (NRC, 1989). Each horse received two meals daily, totaling 3.5 to 4.0 kg, which was one-half to one-third of recommended energy requirements (NRC, 1989). Samples of the feeds were submitted to Dairy One (DHI Forage Testing Laboratory, Ithaca, NY) for proximate and mineral analysis (Table 2).

Trials were conducted in April (3 to 6 wk before foaling), June (3 to 6 wk after foaling), and August (12 to 15 wk after foaling). Each mare was weighed on an electronic scale (Tyrel platform, TC-105, Alweights Hamilton Scale Corp., Richmond, VA) then placed in a stall overnight (12 to 18 h) with free access to water but no feed. A series of blood samples (30 mL drawn into Vacutainer tubes containing sodium heparin, Becton 270, 330, and 390 min after a meal of 1.82 kg of respective feed, PC, SS, or FF. The first sample (baseline) was obtained at 0800, immediately before the feed was given. The SS and PC meals were consumed in 20 to 30 min and the FF in 30 to 40 min. Blood samples were immediately centrifuged at 1,600 × *g* for 10 min, and plasma was removed and stored at –20°C until analysis. Plasma glucose concentrations were determined by the glucose oxidase method using a chemical autoanalyzer (Kit # 442640, Beckman Synchron CX5CE, Brea, CA). Plasma insulin was determined by radioimmunoassay (Coat-A-Count Insulin, kit # TKINX, Diagnostic Products Corp., Los Angeles, CA). Areas under the concentration-time curve (AUC) were calculated as summed trapezoids. Duplicate assays of plasma glucose had an intraassay CV of < 1%, and insulin had a CV of 5.2%. The interassay CV for glucose was 2% and for insulin was 5.5%.

The effects of horse, diet, and stage of the reproductive cycle were evaluated by ANOVA using SAS (SAS Inst. Inc., Cary, NC). In the general linear models procedures, the degrees of freedom were insufficient to run a full model with all interactions. Without interactions, the main effects of horse had *P*-values of 0.28 to 0.72, and the main effects of stage had *P*-values of 0.20 to 0.76. Consequently, a mixed model was applied to early and late lactation with feed and stage as fixed effects and horse as a random effect. Means were compared by the Tukey test with $P < 0.05$.

Results

The FF feed had four times more fat than the SS feed and eight times more fat than PC (Table 2). The NDF was about twice as high and NSC was twice as low in FF than in SS or PC. The CP in the FF was about 12% higher than that in the SS, and there were different levels of phosphorus, magnesium, and potassium between the two feeds.

The horses weighed 669 ± 13.7 kg in April, 583 ± 14.6 kg in June, and 575 ± 16.4 kg in August and maintained a body condition score of 5 to 6 (Henneke et al., 1983). All the horses remained in good health throughout the observational period. No differences were found between any feeds and stages in baseline data for plasma glucose ($P = 0.36$) and insulin ($P = 0.15$); mean concentrations were 74.7 ± 10.9 mg/dL and 5.66 ± 1.80 mIU/L, respectively.

The glucose and insulin curves for the combined PC data of 12 horses are illustrated in Figure 1. The glucose and insulin AUC were the same between experimental groups of horses receiving PC ($P = 0.48$ and $P = 0.34$, respectively), which eliminated the need for using these data as covariates in later experiments. Peaks of plasma glucose and insulin were usually observed in the 90-min samples, although mean values were not different from 60 to 150 min ($P > 0.14$).

Table 2. Nutrient composition of the sugar and starch (SS) and fat and fiber (FF) diets and the pelleted concentrate (PC) feed as analyzed in the DHI Forage Testing Laboratory (Ithaca, NY); data are summarized on a DM basis as means ± SE

	Diet		
Nutrient	SS (n = 12)	FF (n = 12)	PC (n = 3)
DM, %	90.2 ± 0.67^{d}	92.2 ± 0.36^{e}	93.3 ± 1.51^{dv}
DE, Mcal/kg[a]	3.34 ± 0.05	3.50 ± 0.05	3.40 ± 0.01
CP, %	13.7 ± 0.59^{d}	15.4 ± 0.22^{e}	16.92 ± 0.60^{e}
ADF, %	10.7 ± 1.02^{g}	22.6 ± 0.74^{h}	12.70 ± 1.0^{g}
NDF, %	18.5 ± 1.11^{g}	36.9 ± 0.99^{h}	19.9 ± 2.1^{i}
Fat, %	3.2 ± 0.28^{eh}	16.6 ± 0.76^{dg}	1.97 ± 0.20^{fh}
NSC, %[b]	64.6 ± 3.61^{g}	24.7 ± 2.02^{h}	57.4 ± 4.32^{g}
Ash, %[c]	6.70 ± 0.64^{g}	9.74 ± 0.24^{h}	6.82 ± 0.03^{g}
Ca, %	1.15 ± 0.14^{dc}	1.37 ± 0.19^{d}	0.91 ± 0.07^{e}
P, %	0.60 ± 0.05^{f}	1.35 ± 0.06^{d}	0.77 ± 0.02^{e}
Mg, %	0.19 ± 0.01^{g}	0.67 ± 0.03^{h}	0.36 ± 0.02^{i}
K, %	1.07 ± 0.06^{d}	1.28 ± 0.02^{e}	0.87 ± 0.10^{d}
Na, %	0.25 ± 0.03^{gh}	0.22 ± 0.02^{g}	0.012 ± .01^{h}
S, %	0.22 ± 0.01^{de}	0.21 ± 0.01^{d}	0.19 ± 0.01^{e}

[a]DE = 3.6 × NSC + 1.4 × NDF + 8 × fat + 3.6 × CP/(NSC + NDF + fat + CP).
[b]Nonstructural carbohydrate; NSC = 100 − (water + CP + fat + ash + NDF).
[c]Ash = 9.425 × K + 1.442 × P − 4.231 ± 0.867; adjusted r^2 = 0.992, P = 0.004.
[d,e,f]Values with different superscripts are different (P < 0.05).
[g,h,i]Values with different superscripts are different (P < 0.01).

in Figures 2A and 2B, respectively. The glucose responses to PC and SS were similar, except that peak glucose was numerically lower following the SS meal during late lactation than after the PC meal. During all stages, the lowest glucose peak (P < 0.001) and AUC (P < 0.002) was observed after feeding FF, compared to SS and PC, which were similar in glucose peak and AUC.

The effects of feed and stage on peak insulin and insulin AUC are shown in Figures 3A and 3B, respectively. Insulin responses to PC and SS were similar. During all stages, the lowest insulin peak (P < 0.001) and AUC (P < 0.003) was observed after feeding FF compared to SS and PC, which were similar in insulin peak and AUC.

Comparison of responses to feeds during early and late lactation revealed the same pattern of glucose and insulin responses in four sets of data (Figures 2A and 2B, 3A and 3B). The changes between glucose peak and AUC were in opposite directions in the SS and FF groups, and although the stage effect was not significant, interactions were starting to form a trend (P = 0.20, and P = 0.17, respectively). Similar numerical changes in opposite directions were evident for insulin peak and AUC.

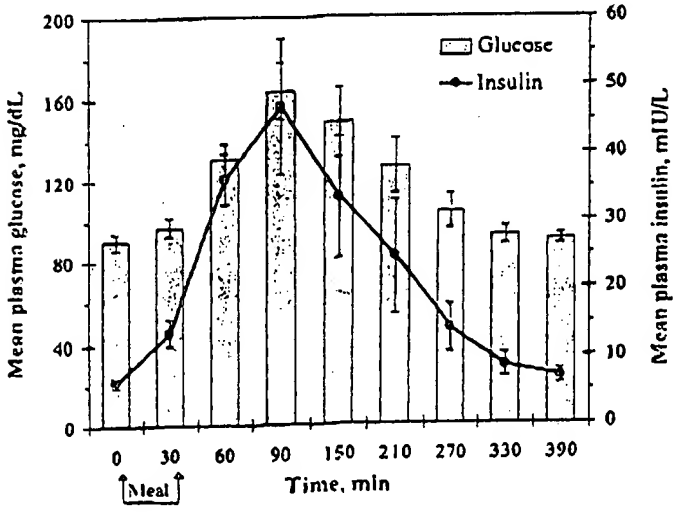

Figure 1. Mean concentration of the plasma glucose (bars) and insulin (solid line) for 12 mares fed the pelleted concentrate (PC) during late gestation. The two groups of mares were combined to illustrate the glucose and insulin curve.

Discussion

Procedurally, these measurements resemble glucose tolerance tests, which give information about the glucose status of the animal, and glycemic indices, which give information about the glucose-equivalents in the diet (Roberts and Hill, 1973; Jenkins et al., 1981). The present glycemic and insulinemic responses are intended to give information on isocaloric meals with differing contents of glucose-equivalents, following previous studies in horses (Stull and Rodiek, 1988; Ralston, 1992; Pagan et al., 1999). In the present experiments, replacement of starch and sugar with fat and fiber moderated the postprandial glycemic and insulinemic effects of a meal fed to lactating mares.

Predictable subsequent metabolic and hormonal changes of the glucose-fatty acid cycle affect free fatty acids, triglycerides, cortisol, thyroid hormones, growth

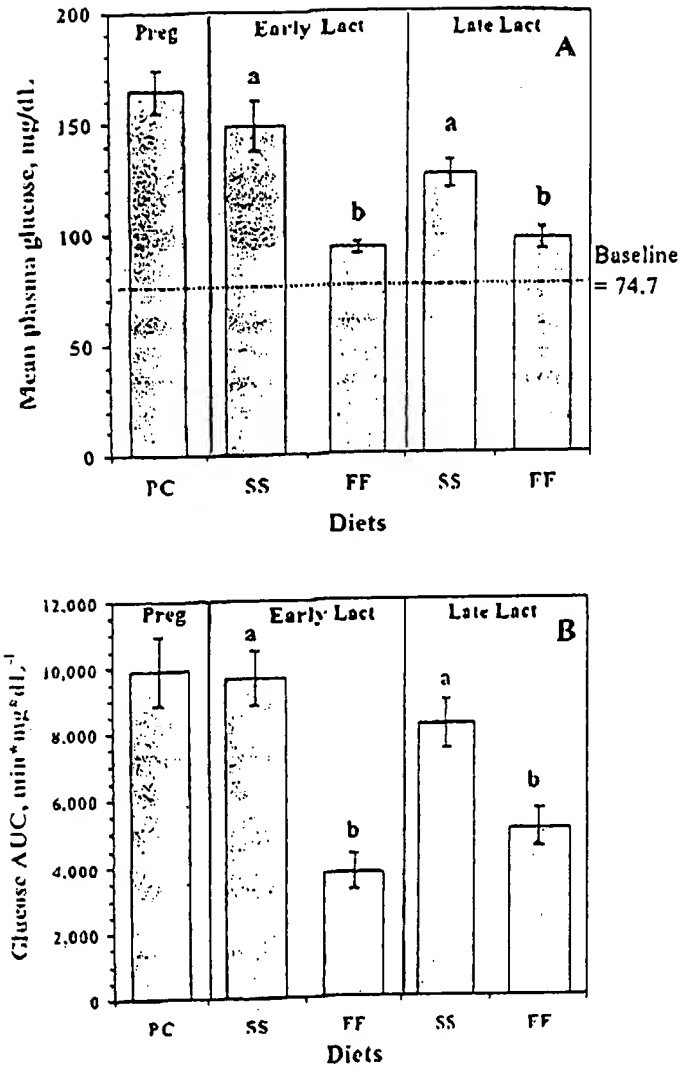

Figure 2. Peak plasma glucose (A) and glucose area under the curve (B) in mares fed the pelleted concentrate (PC) during late gestation (Preg), a feed high in sugar and starch (SS), or a feed high in fat and fiber (FF) during early lactation and late lactation. Groups without a common letter designation differ ($P < 0.05$).

hormone, and insulin-like growth factors (Kronfeld, 1998; Randle, 1998). Daily changes in insulin and counter-regulatory hormones, associated with traditional SS-type concentrate meals, may contribute to the development of certain metabolic disorders (Glade and Reimers, 1985; Clarke et al., 1990; Ralston, 1996). Our results suggest that the metabolic and health impacts are likely to be moderated for meals of FF, which may be more reflective of the nutritional heritage of the horse.

Interpretation of these experiments is complicated in two regards. One involved the possible interaction of the feed and reproductive stage. The other concerned the relative importance of decreasing glucose-equivalents and increasing fat or fiber on the results.

study. Peak glucose and insulin and the AUC of glucose and insulin tended to decrease from early to late lactation in the SS group but to increase in the FF group. Early lactation experiments in the present study encompass the first month after foaling when the milk yield of a mare is at its peak, approximately 11.8 kg/d, or 2.3% of body weight (Gibbs et al., 1982). In contrast, tests in late lactation were performed within the 3rd or 4th mo after foaling, when milk yield has decreased to 10.4 kg/d, or 2.0% of body weight. The feed and stage trend is likely to represent an overall decrease in glucose removal from blood later in lactation, when the rate of glucose removal from blood specifically for milk lactose synthesis and volume production is decreased (Evans, 1971).

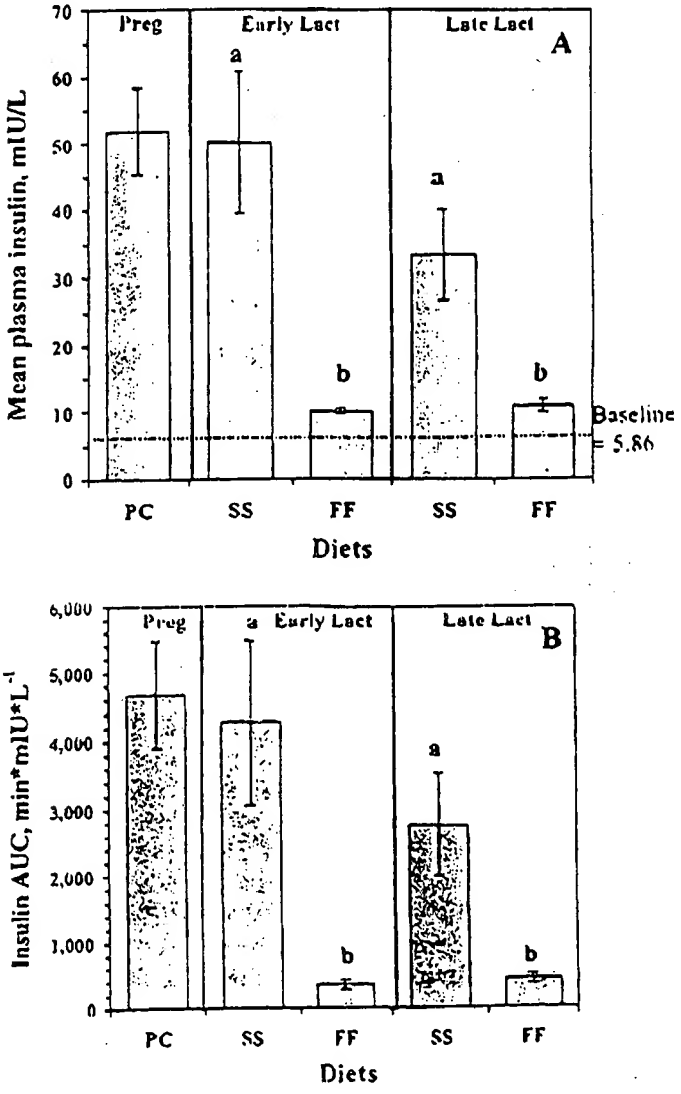

Figure 3. Peak plasma insulin (A) and area under the curve (B) for insulin in mares fed the pelleted concentrate (PC) during late gestation, a feed high in sugar and starch (SS), or a feed high in fat and fiber (FF) during early

The plasma glycemic response to a meal is affected by the meal's composition and the rates of consumption, gastric emptying, hydrolysis yielding glucose, absorption of glucose, and removal of glucose from the blood. Either lowering the glucose-equivalents in the feed or increasing the fat or fiber contents could contribute to changes in the glycemic responses to a meal. Higher fat in the diet may delay or decrease the peak glycemic response by retarding gastric emptying in humans (Wolever, 1990). However, this delay should not affect the AUC. The effect of a high-fat meal on gastric emptying has not been studied in horses to our knowledge. The glucose-equivalents would be approximated by NSC (Table 2), which amounts to 1,045, 1,035, and 339 g in the meals of PC, SS, and FF, respectively. This threefold difference in NSC intake may be compared to the 2.5-fold difference between the SS and FF feeds in glucose AUC early in lactation and the 1.6-fold difference during late lactation.

The increase in plasma insulin concentration reflects an increased rate of insulin release into the blood in response to glucose absorption and hyperglycemia (Mayes, 1996). The subsequent decline probably represents a moderation in the rate of insulin release and an increased rate of insulin removal from blood. The differences in insulin AUC between SS and FF groups were much greater (12-fold and 6-fold in early and late lactation, respectively) than corresponding differences in glucose AUC (154% and 62%, respectively). This finding suggests that the amount of glucose absorption, thus the change in glucose concentration in the plasma, had a greater impact than a certain level of plasma glucose concentration per se on the magnitude of the insulin response.

Several studies have compared the effects of a corn grain meal to those of corn fortified with 10% corn oil. Feeding a meal of alfalfa hay or corn fortified with 10% corn oil produced plasma glucose peaks of 103 and 116 mg/dL, respectively, in horses (Stull and Rodiek, 1988). For meals of corn only or corn and alfalfa (50:50) the glycemic response reached a level of about 142 mg/dL. Another study used six meals fed to horses at maintenance and found smaller differences in peak glucose concentrations that ranged from 99 mg/dL for alfalfa hay to 108 mg/dL for a sweet feed (Pagan et al., 1999). These studies have found various glucose responses ranging from slight variation between different diets to a large range of concentration peaks.

Compared to previous studies, the magnitude of responses in our study was similar for glucose (Stull and Rodiek, 1988; Pagan et al., 1999) but tended to be lower for insulin, especially following a meal of FF (Figure 3). Insulin AUC were about three to five times larger for meals of various grain mixes, compared to an alfalfa meal (Stull and Rodiek, 1988).

The higher fiber content in the FF feed most likely caused a higher production of acetate, propionate, and butyrate in the gut. These can control plasma glucose concentration by acting as a precursor (to propionate) or by sparing glucose oxidation (mainly acetate and betahydroxybutyrate). Such effects are likely to be smaller than that of direct glucose absorption, which may explain the results of several studies that evaluated the effects of feeding hay and other roughages. Peak insulin concentrations were 29 and 49 mIU/L following meals of corn (sevenfold increase from baseline) and corn and alfalfa (12-fold increase), respectively (Stull and Rodiek, 1988). In contrast, plasma insulin increased only 1.6 times following an alfalfa meal. In our study, peak insulin concentrations were 8.7 times baseline for PC and SS, compared to 1.7 times following a FF meal. Thus, the FF feed, which has an energy density similar to that of a typical concentrate, has the insulinogenic effect of a roughage, alfalfa hay.

In practical human dietetics, the glycemic index (GI) is used to design meals for the feeding of athletes before and after an event (Burke et al., 1998) as well as diets for the management of non-insulin dependent diabetics (Brand-Miller and Foster-Powell, 1999). On this basis, foods are classified as low-GI or high-GI. Low-GI meals may be used to reduce the risk of diabetes, along with coronary heart disease and obesity. Consuming a high-GI meal may exaggerate the insulin and glucose responses, which could exacerbate certain metabolic problems. In regard to exercise, high-GI meals consumed after exercise may increase glycogen storage. In contrast, low-GI meals consumed before endurance exercise may prolong glucose availability and enhance performance (Burke et al., 1998).

Similarly, in equine nutrition the circulatory, digestive, metabolic, and hormonal responses to a meal (Clark et al., 1990; Kronfeld, 1998), including the glycemic and insulinemic responses, may be applied to feeding management during and after an athletic event (Stull and Rodiek, 1995) and reducing the risks of gastric ulcers (Murray, 1999), colic (Cohen et al., 1999), osteochondrosis (Ralston, 1996), and laminitis and certain types of rhabdomyolysis (Kronfeld, 1973; Valentine et al., 1998; MacLeay et al., 1999).

Implications

Horse feeds containing abundant starch from grain and sugar from molasses, such as typical pelleted or textured concentrates, have profound metabolic and hormonal effects that may be reduced or eliminated by the replacement of glucose-equivalents with fat and fiber. This exchange of nutrients may be useful in the feeding management of broodmares, and perhaps in reducing the risks of certain digestive and metabolic disorders in horses. However, the difference between the use of these energy sources in mares during different reproductive stages needs to be explored in more detail.

Literature Cited

Brand-Miller, J., and K. Foster-Powell. 1999. Diets with a low glycemic index: From theory to practice. Nutr. Today 34:64–72.

Burke, L. M., G. R. Collier, and M. Hargreaves. 1998. Glycemic index—a new tool in sport nutrition? Int. J. Sport Nutr. 8:401–415.

Clarke, L. L., M. C. Roberts, and R. A. Argenzio. 1990. Feeding and digestive problems in horses. Vet. Clin. N. Am. Equine Pract. 6:483–450.

Cohen, N. D., P. G. Gibbs, and A. M. Woods. 1999. Dietary and other management factors associated with colic in horses. J. Am. Vet. Med. Assoc. 215:53–60.

Evans, J. W. 1971. Effect of fasting, gestation, lactation and exercise on glucose turnover in horses. J. Anim. Sci. 33:1001–1004.

Gibbs, P. G., G. D. Potter, R. W. Blake, and W. C. McMullan. 1982. Milk production of quarter horse mares during 150 days of lactation. J. Anim. Sci. 54:496–499.

Glade, M. J., and T. J. Reimers. 1985. Effects of dietary energy supply on serum thyroxine, triiodothyroine and insulin concentrations in young horses. J. Endocrinol. 104:93–105.

Fowden, A. L., R. S. Comline, and M. Silver. 1984. Insulin secretion and carbohydrate metabolism during pregnancy in the mare. Equine Vet. J. 16:239–246.

Henneke, D R , G. D. Potter, J. L. Kreider, and B. F. Yeates. 1983. Relationship between condition score, physical measurement, and body fat percentage in mares. Equine Vet. J. 15:371–372.

Hoffman, R. M. 1997. Carbohydrate and fat supplementation in grazing mares and foals. Ph.D. dissertation. Virginia Polytechnic Institute and State Univ., Blacksburg.

Hoffman, R. M., and D. S. Kronfeld. 1999. Nutrient requirements for grazing horses: Development of an optimal pasture supplement. In: P. C. Garnsworthy and J. Wiseman (ed.) Recent Advances in Animal Nutrition. Nottingham University Press, Nottingham, U.K.

Hoffman, R. M., D. S. Kronfeld, J. H. Herbein, W. S. Swecker, W. L. Cooper, and P. A. Harris. 1998. Dietary carbohydrates and fat influence milk composition and fatty acid profile of mare's milk. J. Nutr. 128:2706S–2711S.

Holland, J. L., D. S. Kronfeld, and T. N. Meacham. 1996. Behavior of horses is affected by soy lecithin and corn oil in the diet. J. Anim. Sci. 74:1252–1255.

Jenkins, D. J. A., T. M. S. Wolever, R. H. Taylor, H. Barker, H. Fielden, J. M. Baldwin, A. C. Bowling, H. C. Newman, A. L. Jenkins, and D. V. Goff. 1981. Glycemic index of foods: A physiological basis for carbohydrate exchange. Am. J. Clin. Nutr. 34:362–366.

Kronfeld, D. S. 1973. Diet affects performance of racing sled dogs. J. Am. Vet. Med. Assoc. 162:470–472.

Kronfeld, D. S. 1996. Dietary fat affects heat production and other variables of equine performance, under hot and humid conditions. Equine Vet. J. Suppl. 22:24–34.

Kronfeld, D. S. 1998. Clinical Assessment of Nutritional Status of the Horse. In: T. Watson (ed.) Metabolic and Endocrine Problems of the Horse. pp 185–217. W. B. Saunders Company, London.

MacLeay, J. M., S. A. Sorum, S. J. Valberg, W. E. Marsh, and M. D. Sorum. 1999. Epidemiologic analysis of factors influencing exertional rhabdomyolysis in Thoroughbreds. Am. J. Vet. Res. 60:1562–1566.

Mayes, P. A. 1996. Gluconeogenesis and control of the blood glucose. In: J. Dolan and C. Langan (ed.) Harper's Biochemistry. pp 194–204. Appleton and Lange, Stamford, CT.

Murray, M. J. 1999. Gastroduodenal ulceration in foals. Equine Vet. Edu. 11:199–207.

NRC. 1989. Nutrient Requirements of Horses. 5th ed. National Academy Press, Washington, DC.

Oldham, S., G. D. Potter, J. W. Evans, S. B. Smith, T. S. Taylor, and W. S. Barnes. 1990. Storage and mobilization of muscle glycogen in exercising horses fed a fat supplemented diet. J. Equine Vet. Sci. 10:353–359.

Pagan, J. D., P. A. Harris, M. A. P. Kennedy, N. Davidson, and K. E. Hoekstra. 1999. Feed type and intake affect glycemic response in thoroughbred horses. Proc. Equine Nutr. Physiol. Soc. 16:149–150.

Ralson, S. L. 1992. Effect of soluble carbohydrate content of pelleted diets on postprandial glucose and insulin profiles in horses. Pferdeheilkunde 8:112–115.

Ralston, S. L. 1996. Hyperglycemia/hyperinsulinemia after feeding a meal of grain to young horses with osteochondritis dissecans (OCD) lesions. Pferdeheilkunde 12:320–322.

Randle, P. J. 1998. Regulatory interactions between lipids and carbohydrates: The glucose fatty acid cycle after 35 years. Diabetes Metab. Rev. 14:263–283.

Roberts, M. C., and F. W. G. Hill. 1973. The oral glucose tolerance test in the horse. Equine Vet. J. 5:171–173.

Slade, L. M., L. D. Lewis, C. R. Quinn, and M. L. Chandler. 1975. Nutritional adaptation of horses for endurance performance. Proc. Equine Nutr. Soc. 4:114–121.

Stull, C. L., and A. V. Rodiek. 1988. Responses of blood glucose, insulin and cortisol concentrations to common equine diets. J. Nutr. 118:206–213.

Stull, C. L., and A. V. Rodiek. 1995. Stress and glycemic responses to postprandial interval and feed components in exercising horses. J. Equine Vet. Sci. 15:382–386.

Valentine, B. A., H. F. Hintz, K. M. Freels, A. J. Reynolds, and K. N. Thompson. 1998. Dietary control of exercise-induced hyperglycemia in horses. J. Am. Vet. Med. Ass. 212:1588–1593.

Wolever, T. M. S. 1990. The glycemic index. World Rev. Nutr. Diet. 62:120–185.

SOME PHYSIOLOGICAL EFFECTS OF SODIUM BICARBONATE IN DIETS OF YEARLING HORSES[1]

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Summary

A study was made of the physiological effects of adding sodium bicarbonate to growth diets for yearling Quarter Horses. Four treatment groups of four horses each were fed corn-soybean meal-alfalfa based diets for 140 days: (1) control, (2) 1% added $NaHCO_3$, (3) 1% added commercial vitamin-mineral mixture, (4) 1% added $NaHCO_3$ + 1% added vitamin-mineral mixture. Measurements taken every 28 days included individual weights, respiration rates, fecal pH, urine pH; venous pCO_2, pH and packed cell volume; and venous serum sodium, chloride, potassium, calcium, and phosphorus.

Weight changes were not significantly affected by treatments. Increased pH of feeds was associated with increased respiration rates, decreased venous pCO_2, decreased venous pH, increased packed cell volume, increased serum potassium, increased serum phosphorus, and decreased serum chloride. Feed pH had little effect on serum sodium and calcium. Feed pH was positively correlated with urine pH and showed a moderate negative correlation with fecal pH.

Observations suggested that chronic ingestion of 1% sodium bicarbonate was associated with a tendency toward metabolic acidosis, partially compensated by respiratory alkalosis.

Introduction

According to reviews by Baker and Harrison (1979) and Trenkle (1979) some beneficial effects have resulted from the inclusion of sodium bicarbonate in diets of cattle, swine, and poultry. Currently, sodium bicarbonate is being added to some horse diets without researched evidence of its chronic effects.

Acidosis associated with exhaustion was observed in racehorses by Krzywanek (1974). The accumulation of lactic acid may be one of the limiting factors in muscular performance of the horse. Metabolic acidosis also has been associated with severe shock and colic in horses (Rose, 1981). It has been speculated that the addition of sodium bicarbonate to horse diets may ameliorate acidosis.

The purposes of this study were (1) to compare weight changes of yearling Quarter Horses fed similar diets with and without sodium bicarbonate; (2) to assess the physiological effects of chronic ingestion of sodium bicarbonate on the acid-base balance of the horse; and (3) to observe possible interactions between diet supplementation with a commercial vitamin-mineral mixture and addition of sodium bicarbonate.

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Experimental Procedure

Sixteen Quarter Horse yearlings were randomly allotted into four groups of three fillies and one gelding each. All except one purchased filly had similar bloodlines and had been raised in similar environments at the University of Illinois.

Each horse was vaccinated for tetanus and equine encephalomyelitis, and given an intramuscular multivitamin injection providing 1,000,000 U.S.P. units of Vitamin A. Horses were treated for internal parasites just prior to the study and again midway through the study.

All horses were fed the control diet for fourteen days prior to the start of the study (table 1). Thereafter horses were lot-fed their respective test diets for 140 days. Diets were formulated to meet suggested NRC (1978) requirements for yearling horses. Sodium bicarbonate was added at one percent by weight of the total feed to two treatment diets and a commercial vitamin-mineral mixture[1] was added at one percent to two treatment diets, using a 2 x 2 factorial design.

The concentrate portion of the feed was pelletted and was fed at approximately 0900 daily. Hay was fed at 1600. Water was continually available. Test parameters were measured in the morning (0700-1000) prior to feeding, with care taken to minimize excitement and disturbance of horses. Ambient temperatures ranged from 45-85°F (7-29°C) during sampling times during the course of the 140-day study.

Individual weights and respiratory rates were recorded two days apart at the beginning of the study and repeated at 28-day intervals (table 2). Respiratory rates were measured by visual observation of flank movements.

Three venous jugular blood samples were drawn from each horse every 28 days. The first sample of whole blood was collected anaerobically in a heparinized glass syringe, stored in ice, and was analyzed within two hours for pH and pCO_2[2]. Packed cell volume was measured by the microhematocrit technique. The third sample was allowed to clot, centrifuged, and the serum was frozen for subsequent analyses for sodium, chloride, potassium, calcium, and phosphorus concentrations.

Two days after blood collections, individual fecal grab samples and urine specimens were taken and analyzed for pH. To facilitate urine sampling, 2 1/2 cc of furosemide (Lasix) was injected intravenously into each horse.

Feed pellets were analyzed for pH. Digestibilities of test diets are currently being determined.

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[1] Sleek, courtesy of Conagra, Inc., Omaha, NB 68131.
[2] Instrumentation Laboratory Micro 13 Digital pH/Blood gas analyzer.

Results

Feed additives caused marked differences in the pH of the pellets (table 1). The vitamin-mineral pellets (pH 5.780) and the control pellets (pH 6.005) were the most acidic, while the $NaHCO_3$ + vitamin-mineral pellets (pH 7.508) and $NaHCO_3$ pellets (pH7.872) were more alkaline.

Variations in pH of feed were reflected in alterations in several physiological parameters (table 2). Yearlings fed higher pH diets had higher respiration rates. Feed pH was positively correlated with urine pH (corr. coef. = .9723) and was negatively correlated with fecal pH (corr. coef. =-.7588). Venous blood of horses receiving bicarbonate had lower pH and lower pCO_2 values. Average packed cell volumes were greatest for horses fed bicarbonate.

An analysis of venous serum mineral levels showed that increased feed pH was associated with increased potassium, increased phosphorus, and decreased chloride concentrations. Sodium levels appeared unaffected by feed pH but were decreased in the two vitamin-mineral treatments. Calcium concentrations were lower than control values in the $NaHCO_3$ and the vitamin-mineral treatments, while the lowest mean serum calcium level was observed in the $NaHCO_3$ + vitamin-mineral treatment.

Weight gains were not affected by treatments. The larger average daily gain in the vitamin-mineral treatment can probably be attributed to compensatory growth of the purchased filly that began the study in a noticeably thin condition.

Discussion

The high correlation of feed pH with urine pH in this study may be a result of renal compensation for excess absorbed bicarbonate. Elevations in packed cell volume in bicarbonate treatments may be attributed to a diuretic effect resulting in increased water loss in the effort to maintain acid-base balance.

It is thought that the cecum and large intestine of equines are important sites of fiber digestion, and it has been observed that the pH of terminal colon digesta is similer to that of cecal contents (Kern et al., 1974). It may be speculated that the reduced pH of colon digesta in this study may have been associated with a reduced cecal pH, altered microbial populations in the gut and/or reduced digestibility of some feedstuffs. Low fecal pH has been associated with reduced digestibility of starch in cattle fed high concentrate rations (Wheeler and Noller, 1977). Goldberger (1965) suggested that reduced fecal pH may be a result of (1) increased uptake of bicarbonate ions from the gastrointestinal tract, perhaps due to increased activity of carbonic anhydrase; (2) decreased secretion of bicarbonate; and/or (3) increased secretion of hydrogen, chloride, and potassium ions. The alterain fecal pH of this study, however, were not associated with weight changes. Digestibilities of test diets are currently being determined.

The observed trends in venous pH, pCO_2, and respiratory rate are consistent with a tendency toward metabolic acidosis partially compensated by a respiratory alkalosis (Goldberger, 1965; Rose, 1981). These same trends in venous pH and pCO_2 have been observed by Milne (1974) in horses with a compensated metabolic acidosis during exercise. Chronic ingestion of sodium bicarbonate may thus serve to exacerbate acidotic syndromes during exercise, rather than ameliorate them.

Goldberger (1965) and Rose (1981) suggested that metabolic acidosis may occur as a result of (1) increased production and/or retention of acids normally produced in the body, such as sulfuric, phosphoric, and organic acids; or (2) increased loss of base, such as bicarbonate. Goldberger (1965) further observed that carbonic anhydrase in the kidney tubular cells normally acts to conserve bicarbonate in the body. If the activity of this enzyme is inhibited, large amounts of sodium bicarbonate and water are excreted in the urine. This causes a diuresis and an acidosis, due to the loss of bicarbonate. With a rise in urine pH, an increased amount of potassium is lost in the urine due to an exchange mechanism between potassium and hydrogen ions.

Elevated levels of venous serum potassium in horses are often concomitant with acidosis. Normally, 98 percent of body potassium resides intracellularly. It has been noted that when the pH of extracellular fluids decreases, a shift of potassium ions occurs from intracellular to extracellular space. Rose (1981) observed that, for this reason, plasma potassium values tend not to reflect the total body potassium balance. Potassium depletion may occur even while serum levels are normal or elevated. These observations may account for the elevated serum potassium levels in bicarbonate treatments in this study.

The diuretic effect postulated in this study was likely associated with a loss of cations such as sodium, potassium, and calcium ions in the urine. Goldberger (1965) suggested that a resultant drop in serum calcium would cause an elevation in serum inorganic phosphate levels. The observed increase in serum phosphorus in bicarbonate treatments may be due to these effects. The decreased serum chloride levels in horses receiving bicarbonate may have been due to chloride secretion with hydrogen ions into the gastrointestinal tract or an effort to maintain osmotic balance in the presence of continual loss of cations in the urine.

As excess hydrogen ions from an acidosis situation react with absorbed bicarbonate ions in the blood, carbonic acid is formed. This dissociates into water and carbon dioxide. The respiratory center in the medulla and chemoreceptors in the aortic arch and carotid sinus are sensitive to the partial pressure of carbon dioxide and pH of the blood. Yearlings fed sodium bicarbonate in this study probably increased their respiratory rate in an effort to remove excess carbon dioxide through the lungs. This may have resulted in the decreased venous pCO_2 levels observed in these horses, indicative of a tendency toward respiratory alkalosis.

Further research is needed to elucidate the physiological effects of ingestion of sodium bicarbonate by horses, particularly with reference to exercise physiology.

Literature Cited

Baker, D. H., and P. C. Harrison. 1979. Bicarbonate in Poultry and Swine Nutrition. National Feed Ingredients Association, Des Moines.

Goldberger, E. 1965. Water, Electrolyte, and Acid-Base Syndromes. (3rd Ed.). Lea and Febiger, Philadelphia.

Kern, D. L., L. L. Slyter, E. C. Leffel, J. M. Weaver, and R. R. Oltjen. 1974. Ponies vs. steers: microbial and chemical characteristics of intestinal ingesta. J. Anim. Sci. 38:559.

Krzywanek, H. 1974. Lactic acid concentrations and pH values in trotters after racing. J. South African Vet. Assn. 45:355.

Milne, D. W. 1974. Blood gases, acid-base balance and electrolyte and enzyme changes in exercising horses. J. South African Vet. Assn. 45:345.

NRC. 1978. Nutrient Requirements of Domestic Animals, No. 6. Nutrient Requirements of Horses. Fourth Revised Ed. National Academy of Science-National Research Council, Washington, D.C.

Rose, R. J. 1981. A physiological approach to fluid and electrolyte therapy in the horse. Equine Vet. J. 13:7.

Trenkle, A. H. 1979. Sodium Bicarbonate in Beef Nutrition. National Feed Ingredients Association, Des Moines.

Wheeler, W. E., and C. H. Noller. Gastrointestinal tract pH and starch in feces of ruminants. J. Anim. Sci. 44:131.

TABLE 1. COMPOSITION OF DIETS, AS FED, DAILY

Item	Treatments			
	Control	+$NaHCO_3$	+vitamin-mineral mixture	+$NaHCO_3$ +vitamin-mineral mixture
Pellets, total, kg	10.89	11.23	11.23	11.57
Corn, kg	6.79	6.79	6.79	6.78
Soybean meal, kg	3.52	3.52	3.52	3.52
Dried molasses, kg	.51	.51	.51	.51
Iodized salt, kg	.054	.054	.054	.054
Coloring marker, kg	.011	.011	.011	.011
Sodium bicarbonate, kg	--	.338	--	.337
Vitamin-mineral mixture, kg	--	--	.348	.348
Alfalfa hay, kg	21.77	21.77	21.77	21.77
Total feed, kg	32.66	33.00	33.00	33.34
Sodium bicarbonate, percent of total feed	--	1.02	--	1.01
Vitamin-mineral mixture, percent of total feed	--	--	1.05	1.04
Pellets, pH[1]	6.005	7.872	5.780	7.508

[1]Means of 4 samples per treatment.

TABLE 2. PHYSIOLOGICAL EFFECTS OF SODIUM BICARBONATE AND VITAMIN-MINERAL ADDITION TO DIETS OF YEARLING HORSES

Item	Treatments			
	Control	+$NaHCO_3$	+vitamin-mineral mixture	+$NaHCO_3$ +vitamin-mineral mixture
Average daily gain, kg/day[1]	.287	.287	.353	.287
Respiration rate, min^{-1} [2]	11.7	12.6	11.7	12.1
$\bar{v}$ pH[3]	7.4258	7.4105	7.4356	7.4165
$\bar{v}$ pCO_2, mmHg[3]	49.41	48.33	49.07	47.74
Packed cell volume, %[3]	36.8	39.1	37.5	39.4
Serum sodium, Meg/l[3]	138.9	138.0	136.1	137.9
Serum chloride, Meg/l[3]	97.2	96.6	97.0	96.4
Serum potassium, Meg/l[3]	3.94	3.95	3.80	4.04
Serum calcium, mg/dl[3]	12.00	11.90	11.90	11.62
Serum phosphorus, Meg/l[3]	5.35	5.51	5.34	5.60
Urine pH[3]	7.733	7.861	7.667	7.859
Fecal pH[3]	6.853	6.720	6.790	6.595

[1]Means from 4 horses per treatment over duration of study.
[2]Means of 10 periodic samples from each of 4 horses per treatment over duration of study.
[3]Means of 5 periodic samples from each of 4 horses per treatment over duration of study.